SCREENING ON THE FACTOR AFFECTING THE ALKALINE PRETREATMENT OF FIBER PRESSED OIL PALM FROND

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ABSTRACT

Oil palm fronds are a by-product from the harvest of the fresh fruit from oil palms. Nowadays, oil palm fronds are used as a source of roughage for ruminants. Oil palm fronds have lignocelluloses biomass is an organic residue which consists of mainly cellulose, lignin and hemicelluloses, whose basic units are sugars that can be fermented into sugar or other chemicals. The main objective of this research is to screening the factor that affect the alkaline pretreatment in lignin removal. This research was conducted based on two-level factorial design with a total sixteen runs. The studied variables or factors (parameters) e.g. sodium hydroxide concentration, temperature, time pretreatment, lime concentration and ultrasonication were denoted by high level (+1) and low level (-1) where their actual code are 0.50-10%, 40-90ºC, 15-120 minutes, 2.00-10.00% and yes or no, respectively. The oil palm frond biomass with less than 1.0mm were first pre-soaked in mixture of sodium hydroxide and lime solution that have different concentration, then incubated in an microwave with desired temperature and time. For the alkaline-assisted ultrasonic, the same method is approach but by using ultrasonic probe. The OPF was then filtered and washed thoroughly with de-ionized water and dried in oven for 24 hour. Then, the filtrate was weighed. The analysis done using Two-Level Factorial by Design Expert software shows that three factors are affecting with high contribution towards the lignin removal. These factors are the sodium hydroxide concentration, temperature and lime concentration. The factors of NaOH concentration, temperature and lime concentration contributed with 76.30%, 0.50% and 0.29% respectively to the delignification process. In addition, the data obtained was fitted into response which is the amount of lignin removed, Y_L for statistical analysis. Lignin response was found to be significant in terms of model (F-value = 39.79), the regression coefficient, $R^2 = 0.9876$ and adjusted regression coefficient $R_A^2 = 0.9628$.

ABSTRAK

Pelepah kelapa sawit adalah produk daripada tanaman buah-buahan yang segar dari kelapa sawit. Pada masa kini, pelepah kelapa sawit digunakan sebagai sumber pelawas untuk ruminan. Pelepah kelapa sawit mempunyai biojisim lignocelluloses adalah sisa organik yang terdiri daripada terutamanya selulosa, lignin dan hemicelluloses, yang unit asas adalah gula yang boleh diperam ke dalam gula atau bahan kimia lain. Objektif utama kajian ini adalah untuk saringan faktor yang memberi kesan kepada prarawatan alkali dalam penyingkiran lignin. Untuk mencapai matlamat tersebut, kajian ini telah dijalankan di dijalankan berdasarkan reka bentuk faktorial dua tingkat dengan jumlah enam belas experiment. Pembolehubah yang dikaji atau faktor-faktor (parameter) seperti kepekatan natrium hidroksida, suhu, masa prarawatan, kepekatan kapur dan ultrasonikasi telah ditandakan oleh tahap tinggi (1) dan tahap rendah (-1) di mana kod sebenar mereka iaitu 0,50-10%, 40 -90ºC, 15-120 minit, 2,00-10,00% dan ya atau tidak. Biojisim pelepah kelapa sawit dengan kurang daripada 1.0mm direndam dalam campuran natrium hidroksida dan larutan kapur yang mempunyai kepekatan yang berbeza, kemudian dieram dalam microwave dengan suhu dan masa yang dikehendaki. Untuk ultrasonik, kaedah yang sama digunakan tetapi dengan menggunakan probe ultrasonik. Pelepah sawit kemudian ditapis dan dibasuh sehingga bersih dengan air dan dikeringkan dalam ketuhar selama 24 jam. Kemudian, berat ditimbang. Analisis dilakukan dengan menggunakan dua Level faktorial oleh perisian Design Pakar menunjukkan bahawa tiga faktor yang mempengaruhi dengan sumbangan yang tinggi ke arah penyingkiran lignin. Faktor-faktor adalah kepekatan natrium hidroksida, suhu dan kepekatan kapur. Faktor-faktor kepekatan NaOH, suhu dan kepekatan kapur menyumbang dengan 76,30%, 0.50% dan 0.29% masing-masing menyumbang kepada proses penyingkiran lignin itu. Di samping itu, data diperolehi daripada Design Expert telah dipasang ke dalam tindak balas, jumlah lignin, YL untuk analisis statistik. Sambutan Lignin didapati ketara dari segi model (F-value = 39,79), pekali regresi, R^2 = 0,9876 dan diselaraskan pekali regresi R_A^2 = 0.9628.

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Malaysia is one of the world's top producer of oil palm product. The oil palm fronds (OPF) have the great potential in satisfying the energy demand due to its abundant availability. Commonly, wastes from oil palm crops are used as animal feed but this is not the optimal economically beneficial way of manipulating the wastes. After harvesting, the wastes are usually burned or left to rot in the field. In addition, Malaysia is known as the world's leading palm oil producer and exporter, accounting for about 47% of global palm oil production and 89% of exports (Sumathi *et al*, 2008). In 2007 alone, Malaysia was reported to produce approximately 38,256 dry kton of oil palm lignocellulosic waste, with 44% comprised of OPF (Goh *et al*, 2010).

Lignocellulosic biomass is one of the most available and renewable resources which represent a promising low cost raw material for the production of biofuel, bioenergy and added value biomolecules. The major chemical components of lignocellulosic biomass lignocellulosic biomass are cellulose, hemicellulose and lignin. Cellulose is a linear polymer of anhydroglucopyranose units linked by ether bonds. Hemicellulose as cellulose, are polymers constituted of sugar units. They differ from cellulose by being smaller and branched polymers usually containing more than one sugar type; they are also amorphous polysaccharides. Lignin is a complex, crosslinked, three dimensional polymer formed with phenylpropane units .

During biomass pre-treatment lignocellulosic biomass is pre-treated with acids or enzymes in order to reduce the size of the feedstock and to open up the plant structure. Normally, the structure of cellulosic biomass is altered; lignin seal is broken,

hemicelluloses is reduced to sugar monomers, and cellulose is made more accessible to the hydrolysis that convert the carbohydrates polymers into fermentable sugars. Then continue with enzymatic hydrolysis. This is a chemical reaction that releases sugars, which are normally linked together in complex chains. In early biomass conversion processes, acids were used to accomplish this (Chin *et al*, 2013).

1.2 Problem Statement

In the world today, biomass is became a massive problem to many country due to its causes to environment. Many studies have been made by several scientist but not all them can solve the problem of biomass. Utilization of waste material such as palm frond also being studied. The race for producing biodegradable products has increase tremendously. Different approaches have been attempted to use biomass as natural biopolymer for production of biodegradable plastics. It is almost 26.2 million tonnes of oil palm fronds in Malaysia according to MARDI. This biomass has been the main source cellulose fiber. This research is important because of Malaysia total net of importing cellulose is more than RM300 million per year and keep increasing. So, this research will help palm oil sector to solve the biomass problem of oil palm frond and turn them into valuable product which is cellulose fiber.

At present, Malaysia is the largest exporter of palm oil in the international market. In the process of extraction of palm oil from oil palm fruit, a lignocellulosic material oil palm empty fruit bunch (OPEFB) and palm frond is generated as a waste product. Approximately 15 million tons of OPEFB and frond biomass waste is generated annually throughout Malaysia by palm oil mills. At present Malaysia is the largest exporter of palm oil in the international market. In the process of extraction of palm oil from oil palm fruit, a lignocellulosic material oil palm empty fruit bunch (OPEFB) and palm frond is generated as a waste product. Approximately 15 million tons of OPEFB and frond biomass waste is generated annually throughout Malaysia by palm oil mills. Lignocellulose is the major structural component of woody and herbaceous plants such as oil palm tree. It represents a major source of renewable organic matter. Lignocellulose consists of lignin, hemicellulose, and cellulosic material. The chemical properties of the components of lignocellulose make them a substrate of

enormous biotechnological value. Much of the lignocellulosic wastes are disposed by biomass burning, which is not restricted to developing countries alone, but it is considered a global phenomenon. In addition, the problem arises when all of this biomass is not being treated and left to rot in the plantations to provide some nutrient. Unfortunately, these wastes may create environmental problems due to accumulation of high organic content. Therefore, environmental management is placing greatest emphasis in waste minimisation at source or recycling. Moreover, a growing awareness of the "need not to pollute" has forced this industry to look more closely at the milling operation. It is recommended to treat and manipulate the waste to produce useful product.

1.3 Objective

The objective of this research is to screening the factor affecting the alkali pretreatment in lignin removal by using Design of Expert.

1.4 Scope

i. To investigate the effect of the key parameters for alkaline pretreatment in lignin removal. These parameters include:

A - reaction temperature (40-90°C)

B - NaOH concentration (0.5-10%)

C - pretreatment time (15-120 mins)

D - lime concentration (2-10%)

E - Ultrasonication (yes/no)

CHAPTER 2

LITERATURE REVIEW

2.1 Lignocellulose Biomass

Lignocellulosic biomass which is produced from the oil palm industries include oil palm trunks (OPT), oil palm fronds (OPF), empty fruit bunches (EFB) and palm pressed fibres (PPF), palm shells and palm oil mill effluent palm (POME) (F.Sulaiman, 2010).

2.2 Oil Palm Biomass

Oil palm is the most important product of Malaysia that has helped to change the scenario of its agriculture and economy. Despite the obvious benefits, oil palm mill also significantly contributes to environmental degradation, both at the input and the output sides of its activities. On the input side, crude palm oil mills use large quantities of water and energy in the production processes, and on the output side, manufacturing processes generate large quantities of solid waste, wastewater and air pollution. The solid wastes may consist of empty fruit bunches (EFB), mesocarp fruit fibers (MF) and palm kernel shells (PKS). The liquid waste is generated from an extraction of palm oil of a wet process in a decanter. This liquid waste combined with the wastes from cooling water and sterilizer is called palm oil mill effluent (POME). During POME digestion, odor released into surrounding air, thus, reduces air quality in the surrounding lagoons area. Disposal of EFB into oil palm plantation without recovering remnant oil in the EFB contributes to oil spills. Incineration of EFB means wasting renewable energy source and heat which actually could be provided for boiler in palm oil mill. At present, PKS and MF wastes are used extensively as fuel for steam production in palm-oil mills.

EFB is a resource which has huge potential to be used for power generation, currently not being utilized. The application of shells for road hardening has no impact to the environment, however, current practice is actually wasting potential renewable energy source. Methane gas is one among other green house gases which can cause ozone depletion. However, at present, methane in biogas generates during POME digestion is not being utilized or captured and it just escapes into the atmosphere. Palm oil mill residues are currently underutilised; therefore, maximizing energy recovery from the wastes is desirable for both economic and environmental reasons. Oil palm waste is a reliable resource because of its availability, continuity and capacity for renewable energy solution. Furthermore, in current situation the presence of oil palm wastes has created a major disposal problem, thus, affect the environmental. The technological, economic, energy balance, and environmental considerations must be kept at a balance to meet the best solution of utilization oil palm wastes. There is abundance of raw materials available of the palm tree consisting of around 90% of biomass wastes and only around 10% of oil. About 90 million tonnes of oil palm fruit production was recorded in 1998; however, 43-45% of this was mill residues in the form of EFB, shell and fibre. Palm fronds and stems are currently underutilised, and the presence of these oil palm wastes has created a major disposal problem. Therefore, maximising energy recovery from the wastes is desirable for both the environmental and economic reasons. Direct combustion, gasification, pyrolysis, liquefaction, fermentation and anaerobic digestion are alternate conversion technologies available to maximise energy recovery. Therefore, sustainable development can be promoted by encouraging energy projects for the long term, utilising local skills and creating employment (Sulaiman, 2008)

2.2.1 Oil Palm Frond

Among the palm oil biomasses produced from Malaysia's palm oil industry, OPF was chosen as the feedstock in this study mainly due to the abundance of OPF generated during the harvesting of fresh fruit bunches. Approximately 51 million tons of OPF was produced in 2008, accounting for 53% of the total palm biomass (Goh *et al*, 2010). Unlike other palm biomass, such as palm shells, palm fibres, palm kernels, palm trunks

and empty fruit bunches, OPFs are still under-utilised and discarded on the plantation (Tan *et al*, 2011).

Figure 2-1: Example of raw material of oil palm frond

2.2.2 Lignocellulose component in Lignocellulose Biomass

Lignocellulosic materials consist mainly of three polymers which is cellulose, hemicellulose and lignin along with smaller amounts of pectin, protein, extractives (soluble nonstructural materials such as nonstructural sugars, nitrogenous material, chlorophyll, and waxes), and ash. Typically, most of the agricultural lignocellulosic biomass is comprised of about 10-25% lignin, 20-30% hemicellulose, and 40-50% cellulose. The composition of these constituent can vary from one plant species to another. Analysis result of oil palm raw material is shown in Table 2-1. Table 2-1 showed that cellulose content in oil palm's raw material is higher than others component. Higher cellulose content in the sample can produce higher glucose, however hemicellulose content can also hydrolyze.

Component	**Frond**	**Trunk**	**Fiber**	**Shell**	**EFB**
Cellulose (wt.%)	31.0	39.9	19.0	14.7	35.8
Hemicellulose (wt.%)	17.1	21.2	15.2	16.4	21.9
Lignin (wt.%)	22.9	22.6	30.5	53.6	17.9
Wax (wt.%)	2.0	3.1	9.1	2.3	4.0
Ash (wt.%)	2.8	1.9	7.0	2.3	3.0
Others(by difference)	24.2	11.3	19.2	10.7	17.4

Table 2-1: Chemical compound content of oil palm raw material

2.2.2.1 Lignin

Lignin is the most abundant polymers in nature after cellulose and hemicellulose and is present in the cellular wall. It is an amorphous heteropolymer consisting of that are held together by different kind of three different phenylpropane units (p-coumaryl, coniferyl and sinapyl alcohol) linkages. The main purpose of lignin is to give the plant structural support, impermeability, and resistance against microbial attack and oxidative stress. The amorphous heteropolymer is also non-water soluble and optically inactive; all this makes the degradation of lignin very tough. Lignin, just like hemicellulose, normally

starts to dissolve into water around 180 _C under neutral conditions. The solubility of the lignin in acid, neutral or alkaline environments depends however on the precursor (p-coumaryl, coniferyl, sinapyl alcohol or combinations of them) of the lignin (A.T.W.M.Hendriks, 2008).

Lignin is hard to be degradabled both of chemically and enzymatically. Soft wood contains more lignin than hard wood and mostly agriculture residue. Since there is chemical bonding between lignin and hemicellulose in fermentation so it makes lignocellulose to be resistant for chemical and biological degradation (MJ, 1999). Lignin is an amorphous heteropolymer network of phenyl propane units (p-coumaryl, coniferyl and sinapyl alcohol) held together by different linkages (Hendricks *et al*, 2009). Lignin is generally accepted as the'glue' that binds the different components of lignocellulosic biomass together, thus making it insoluble in water. Because of its close association with cellulose microfibrils, lignin has been identified as a major deterrent to enzymatic and microbial hydrolysis of lignocellulosic biomass. Chang and Holtzapple, (Chang *et al*, 2000) showed that biomass digestibility is enhanced with increasing lignin removal. In addition to being a physical barrier, the detrimental effects of lignin include:

i) nonspecific adsorption of hydrolytic enzymes to "sticky" lignin;
ii) interference with, and non-productive binding of cellulolytic enzymes to lignin-carbohydrates complexes; and
iii) toxicity of lignin derivatives to microorganisms. Different feedstocks contain different amount of lignin that must be removed via pretreatment to enhance biomass digestibility.

The lignin is believed to melt during pretreatment and coalesces upon cooling such that its properties are altered; it can subsequently be precipitated. Delignification (extraction of lignin by chemicals) causes biomass swelling, disruption of lignin structure, increases in internal surface area, and increased accessibility of cellulolytic enzymes tocellulose fibers. Although not all pretreatments result in substantial delignification, the structure of lignin may be altered without extraction due to changes in the chemical properties of the lignin. The pretreated biomass becomes more digestible than the raw biomass even though it may have approximately the same lignin content as non-pretreated biomass (Agbor *et al*, 2011).

2.2.2.2 Hemicellulose

Hemicellulose is a complex carbohydrate structure that consists of different polymers like pentoses (like xylose and arabinose), hexoses (like mannose, glucose and galactose), and sugar acids. Hemicellulose is the second most abundant polymer (20–50% of lignocellulose biomass) and differs from cellulose in that it is not chemically homogeneous. Hemicelluloses are branched, heterogenous polymers of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose) and acetylated sugars. They have lower molecular weight compared to cellulose and branches with short lateral chains that are easily hydrolysed (Agbor et al, 2011). Hemicelluloses differ in composition. Among the key components of lignocellulosics, hemicelluloses are the most thermo-chemically sensitive. Hemicelluloses within plant cell walls are thought to 'coat' cellulose-fibrils and it has been proposed that at least 50% of hemicellulose should be removed to significantly increase cellulose digestibility. Nevertheless, severity parameters must be carefully optimized to avoid the formation of hemicellulose degradation products such as furfurals and hydroxymethyl furfurals which have been reported to inhibit the fermentation process (Agbor et al., 2011).

2.2.2.3 Cellulose

Cellulose is the main constituent of plant cell wall conferring structural support and is also present in bacteria, fungi, and algae. These fibrils are attached one to another by hemicellulose, amorphous polymer from different sugar like other polymers, such as pectin and closed by lignin. When existing as unbranched, homopolymer, cellulose is a polymer of ß-D-glucopyranose moieties linked via ß-(1,4) glycosidic bonds with well documented polymorphs. The repeating unit of the cellulose chain is the disaccharide cellobiose as oppose to glucose in other glucan polymers. The cellulose chains (20–300) are a grouped together to form microfibrils, which are bundled together to form cellulose fibres. The cellulose microfibrils are mostly independent but the ultrastructure of cellulose is largely due to the presence of covalent bonds, hydrogen bonding and Van der Waals forces. Hydrogen bonding within a cellulose microfibril determines

‘straightness’ of the chain but interchain hydrogen bonds might introduce order (crystalline) or disorder (amorphous) into the structure of the cellulose (Agbor et al., 2011).

2.3 Application of Lignocellulose Component

Lignocellulose is the major structural component of woody plants and non-woody plants such as grass and represents a major source of renewable organic matter. Lignocellulose consists of lignin, hemicellulose and cellulose. The chemical properties of the components of lignocellulosics make them a substrate of enormous biotechnological value. Large amounts of lignocellulosic “waste” (Table 2-2) are generated through forestry and agricultural practices, paper-pulp industries, timber industries and many agro industries and they pose an environmental pollution problem (Howard *et al*, 2003). The huge amounts of residual plant biomass considered as “waste” can potentially be converted into various different value added products including biofuels, chemicals, cheap energy sources for fermentation, improved animal feeds and human nutrients. Lignocellulytic enzymes also have significant potential applications in various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper, and agriculture (Howard *et al*, 2003).

Table 2-2 : Types of lignocellulosic materials and their current uses

Lignocellulosic material	Residues	Competing use
Grain harvesting Wheat, rice, oats barley and corn	Straw, cobs, stalks, husks,	Animal feed, burnt as fuel, compost, soil conditioner
Processed grains Corn, wheat, rice, soybean	Waste water, bran,	Animal feed
Fruit and vegetable harvesting	Seeds, peels, husks, stones, rejected whole fruit and juice	Animal and fish feed, some seeds for oil extraction
Fruit and vegetable processing	Seeds, peels, waste water, husks, shells, stones, rejected whole fruit and juice	Animal and fish feed, some seeds for oil extraction
Sugar cane other sugar products	Bagasse	Burnt as fuel
Oils and oilseed plants Nuts, cotton seeds, olives, soybean etc.	Shells, husks, lint, fibre, sludge, presscake, wastewater	Animal feed, fertiliser, burnt fuel
Animal waste	Manure, other waste	Soil conditioners
Forestry-paper and pulp Harvesting of logs	Wood residuals, barks, leaves etc.	Soil conditioners, burnt
Saw-and plywood waste	Woodchips, wood shavings, saw dust	Pulp and paper industries, chip and fibre board
Pulp & paper mills	Fibre waste, sulphite liquor	Reused in pulp and board industry as fuel
Lignocellulose waste from communities	Old newspapers, paper, cardboard, old boards, disused furniture	Small percentage recycled, others burnt
Grass	Unutilised grass	Burnt

Biomass can be considered as the mass of organic material from any biological material, and by extension, any large mass of biological matter. A wide variety of biomass resources are available (Table 2-2) on our planet for conversion into bioproducts. These may include whole plants, plant parts (e.g. seeds, stalks), plant constituents (e.g. starch, lipids, protein and fibre), processing byproducts (distiller's grains, corn solubles), materials of marine origin and animal byproducts, municipal and industrial wastes (Howard *et al*, 2003). These resources can be used to create new biomaterials and this will require an intimate understanding of the composition of the raw material whether it is whole plant or constituents, so that the desired functional elements can be obtained for bioproduct production.

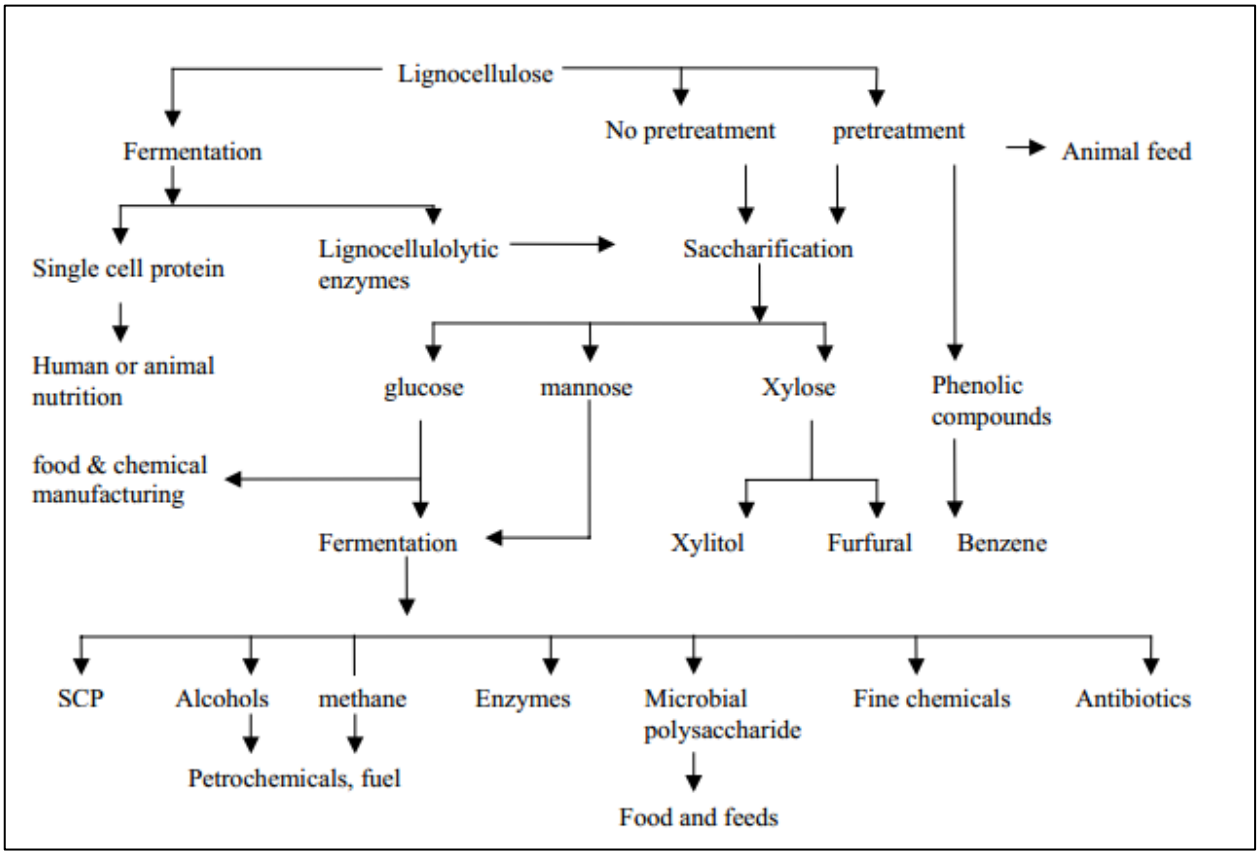

Figure 2-2: Generalised process stages in lignocellulose bioconversion into value-added bioproducts (Anwar *et al*, 2014).

One potential method for the low cost production of bio-ethanol is to utilize the lignocellulosic or agro-industrial biomass because they contain carbohydrates that must be first converted into simple sugars (glucose) and then fermented into ethanol. Given this reality, nations around the world are investing in alternative sources of energy, including bio-ethanol. The conversion of lignocellulosic biomass into higher value added products like fine chemicals or bio-fuel production normally requires a multi-step processing that include (i) pre-treatment (mechanical, chemical, or biological etc) (ii) enzymatic hydrolysis (iii) fermentation process (Anwar *et al*, 2014). Figure 2-2 illustrating a thermo-mechanical and biochemical processing of lignocellulosic biomass into various values added biotechnological products.

2.4 Pretreatment of Lignocellulose Biomass

Pretreatment is an important tool for practical cellulose conversion processes. Pretreatment is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars as represented in the schematic diagram of Figure 2-3. Goals of Pretreatment. The beneficial effects of pretreatment of lignocellulosic materials have been recognized for a long time. The goal of the pretreatment process is to remove lignin and hemicellulose, reduce the crystallinity of cellulose, and increase the porosity of the lignocellulosic materials. Pretreatment must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by hydrolysis, (2) avoid the degradation or loss of carbohydrate, (3) avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes, and (4) be cost-effective (Kumar *et al*, 2009).

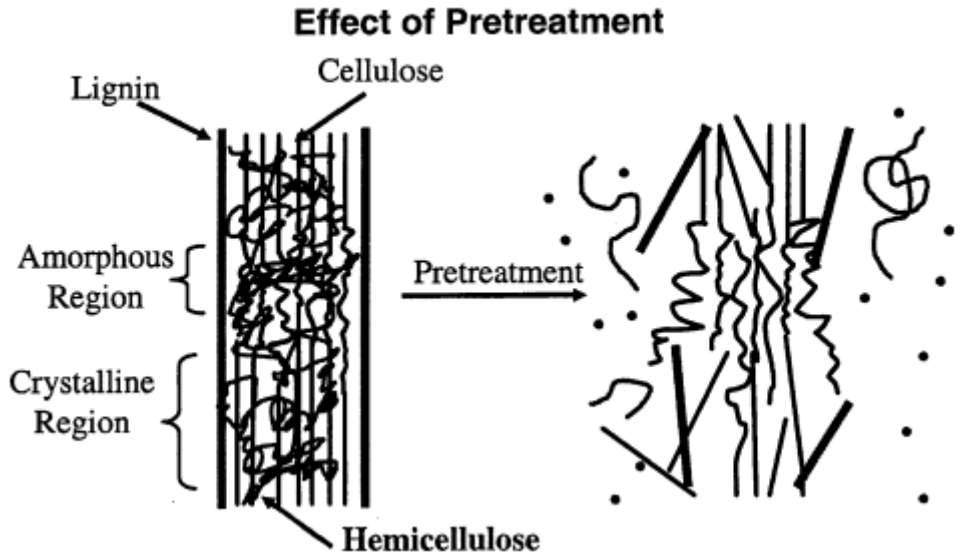

Figure 2-3: The structure of lignin before and after the pretreatment

2.4.1 Type of Pretreatment

2.4.1.1 Physical Pretreatment

There are a number of key features for the effective pretreatment of lignocellulosic biomass. The pretreatment process should have a low capital and operational cost. It should be effective on a wide range and loading of lignocellulosic material and should result in the recovery of most the lignocellulosic components in a useable form in separate fractions (Valery B. Agbor *et al*, 2011). Many methods were extensively used in the world in order to remove lignin from lignocellulosic biomass molecule. There were physical, chemical and biological processes. The purpose of pretreatment is to remove lignin and hemicellulose, reduce crystallinity, and increase the porosity of the materials (Sun *et al*, 2008). Formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes, and be cost-effective (Sun et al, 2008). Mechanical comminution and pyrolysis is an example of physical pretreatment. Waste materials can be comminuted by a combination of chipping, grinding and milling to reduce cellulose crystallinity. The size of the materials is usually 10–30 mm after chipping and 0.2–2 mm after milling or grinding (Sun *et al*, 2008). Vibratory ball milling has been found to be more effective in breaking down the cellulose crystallinity of spruce and aspen chips and improving the digestibility of the biomass than ordinary ball milling. The power requirement of mechanical comminution of agricultural materials depends on the final particle size and the waste biomass characteristics (Sun *et al*, 2008). Pyrolysis is when the material is treated in temperature greater than 300 oC (Sun *et al*, 2008).

2.4.1.2 Chemical Pretreatment

There are many chemical pretreatment technique used in the industry nowadays. The techniques are such as ozonolysis, acid hydrolysis, and alkaline hydrolysis. Alkaline treatments, ozonolysis, peroxide and organosolv treatments are some of the methods usually employed for lignin removal from lignocellulose biomass. Such methods are effective for lignin solubilization but in most of them, part of the hemicellulose is also hydrolyzed (Choi *et al*, 2013). Ozonation has been widely used to reduce the lignin

content of both agricultural and forestry wastes (Balat, 2011) .Ozone can be used to degrade lignin and hemicelluloses in many lignocellulosic materials such as wheat straw, bagasse, green hay, peanut, pine, cotton straw, and poplar sawdust (Sun *et al*, 2008). The advantage of ozonolysis is it effectively removes lignin, it does not produce toxic residues for the downstream processes, and the reactions are carried out at room temperature and pressure. However, a large amount of ozone is required, making the process expensive (Sun *et al*, 2008). Acid hydrolysis uses concentrated acid such as sulphuric acid and hydrochloric acid to treat lignocellulosic materials. Although they are powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive and hazardous and require reactors that are resistant to corrosion. In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible (Sun *et al*, 2008). Alkaline hydrolysis uses bases to treat lignocellulosic materials and the effect depends on the lignin content of the materials (Sun *et al*, 2008). The mechanism of alkaline hydrolysis is believed to be saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components, for example, lignin and other hemicellulose. The porosity of the lignocellulosic materials increases with the removal of the crosslinks (Sun *et al*, 2008). Dilute NaOH treatment of lignocellulosic materials caused swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure (Sun *et al*, 2008). The disadvantage of alkaline hydrolysis is it require long residence time, it form irrecoverable salts and incorporated into biomass (Balat, 2010). Alkali treatments refers to the application of alkaline solutions such as NaOH, Ca(OH)2 or ammonia. Among these, treatment with NaOH is one of the most used for delignification of agricultural residues. The alkali treatment causes swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure. As a consequence, the lignin is dissolved from the raw material, being separated in the form of a liquor rich in phenolic compounds that represents the process effluent. (Choi *et al*., 2013).

2.4.2 Factors affecting Alkaline Pretreatment

Alkaline pretreatment of lignocelluloses with NaOH can remove or modify its lignin by fracturing the ester bonds that form cross-links between xylan and lignin, thereby increasing the porosity of the biomass. The process is very complicated, involving several reactive and nonreactive phenomena, e.g. dissolution of nondegraded polysaccharides, peeling-off reactions, hydrolysis of glycosidic bonds and acetyl groups and decomposition of dissolved polysaccharides (Fengel, 1984). Therefore, the efficiency of NaOH pretreatment depends on the process conditions, e.g. temperature, concentration of NaOH, and treatment time, as well as the inherent characteristics of the lignocellulose used (Zhao, 2008).

NaOH reaction on pretreatment causes the OPF surface to be destroyed and the cell internal structure exposed, generating some irregular cracks and pores. Moreover, the lignocellulosic biomass becomes "thinner and striated" under the SEM analysis after the materials had been pretreated in alkali. It is believed that the lignin has degraded, hence increases the exposure of cellulose and hemicelluloses in the lignocelluloses biomass to hydrolyzing enzymes such as xylanase. The alteration of structure increases the external surface area and the porosity of the pretreated OPF, thus enhancing the enzymatic attacks in breaking down the complex sugar into simpler sugar or reducing sugar (Sabrina *et al*, 2014).

2.4.2.1 Factor A (NaOH concentration)

Pretreatment of lignocellulosic biomass with alkali such as NaOH can eradicate or modify the lignin content by rupturing the ester bonds that form cross links involving xylan and lignin, thus increasing the porosity of the lignocelluloses (Preeti B. Subhedar *et al*, 2014). NaOH pretreatment processes into "high concentration" and "low concentration" processes. In low-NaOH concentration processes, typically 0.5-4% NaOH at high temperature and pressure is used and no recycling of NaOH occurs. Its mechanism is reactive destruction of lignocelluloses, while NaOH at high temperatures

disintegrates the lignin and hemicellulose and removes them from the solid phase. On the other hand, in high concentration NaOH pretreatment, usually 6 to 20% NaOh is used at ambient pressure and low temperatures. Lignin is not significantly removed from the cellulose. (Mirahmadi *et al*, 2010).

2.4.2.2 Factor B (Temperature)

NaOH concentration and pretreatment time also are significant in increasing the hemicelluloses content instead of the temperature itself. Hemicelluloses content was significantly higher at lower NaOH concentration and diminished.

2.4.2.3 Factor C (Pretreatment Time)

According to (Rogalinski, 2008) ,the longer reaction time leads to the solubilization of biomass into glucose which then further degraded into smaller compounds such as furfural. The existence of other compounds will initiates the inhibition factors in the enzymatic hydrolysis and retards the production of reducing sugar. Therefore, an adequate time should be given during the pretreatment to increase the accessibility of cellulose by enzymes. Increased of NaOH concentration in the pretreatment assists the increase of surface area and the formation of pores, hence permitting easier enzyme access and attacks on carbohydrates for reducing sugar production.

2.4.2.4 Factor D (Lime pretreatment)

Sodium hydroxide (NaOH) and lime (Ca(OH)2) were innovatively used together in this study to improve the cost-effectiveness of alkaline pretreatment of oil palm frond at ambient temperature. Based on the properties of NaOH and lime, it is possible to manipulate the usage of these two alkali reagents to make them work together to

achieve a cost-effective pretreatment. First, lime is much cheaper than NaOH, thus being able to replace part of the NaOH alkalinity at a very low cost. Second, due to its poor solubility, a significant part of lime exists as solid and would gradually dissolve to supplement the alkalinity consumed by the biomass, thus stabilizing the pH at a high level throughout the pretreatment. Third, calcium ions, each carrying two positive charges, is expected to provide linkages within the biomass which are negatively charged at alkaline conditions due to the ionization of some functional groups including carboxyl, methoxy, and hydroxyl, thus preventing serious solid loss which is commonly observed in NaOH pretreatment. Therefore, lime, although not strong enough by itself, can be used as a supplementary reagent to strong but expensive NaOH to improve the economic promise of alkaline pretreatment at ambient temperature (Xu & Cheng, 2011)

2.4.2.5 Factor E (Ultrasonication)

The alkaline pretreatment process can be improved further by the application of ultrasound. The present study conjugates alkaline pretreatment with ultrasound irradiation. The ultrasonic treatment of aqueous media produces cavitation, which generates conditions of high temperature, pressure and extreme shear forces (Preeti B. Subhedar *et al*, 2014). Ultrasonication has seen wide application in cell crushing, removal of dead cell in surgery and breaking filamentous algae in small pieces. Sonication is the act of applying ultrasound energy to agitate the particles and to speed up dissolution of molecules by breaking the intermolecular interaction (Jagdish Gabhane *et al*, 2014). The application of ultrasound for delignification of lignocellulosic matrix is still in emerging stage. Ultrasound assisted fractionation of the lignocellulosic biomass improves the effectiveness of the classical treatments such as alkaline treatment, giving proportionately higher yield and selectivity of the obtained products. Ultrasound treatment shortens the processing time as well as reduces the requirement of alkali. The main limitations of the alkaline pretreatment of lignocellulosic biomass include relatively longer pretreatment time and consumption of more alkali (Preeti B. Subhedar *et al*, 2014).